

BACKCROSS ASSISTED BY RAPD MARKERS FOR THE INTROGRESSION OF ANGULAR LEAF SPOT RESISTANCE GENES IN COMMON BEAN CULTIVARS

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Angular leaf spot of common bean is caused by the fungal pathogen *Phaeoisariopsis griseola* (Sacc.) Ferraris and occurs in bean production areas in Brazil. Cultivars Mexico 54 and Mar 2 are used as donor parents in the backcross breeding program of BIOAGRO/UFV (Minas Gerais, Brazil) to create bean cultivars resistant to this disease. Mexico 54 is resistant to 20 and Mar 2 to 16 races of *P. griseola* identified in Brazil (Nietshe, 2000). The recurrent parent, Rudá, is a “carioca” type cultivar, with good yield potential but susceptible to most races of angular leaf spot.

In this work we identified homozygous resistant bean lines genetically closer to the recurrent parent using the RAPD technique. Molecular markers linked to the resistance genes were also used to ensure that these genes were maintained during the breeding process.

A BC₂F₃ population was obtained from the cross Ruda vs Mexico 54 and a BC₁F₃ population from Ruda vs Mar 2. All crosses were done in the greenhouse under controlled environmental conditions. After each backcross and selfing the plants were inoculated with spore suspensions of *P. griseola* (2×10^4 spores/ml) and incubated for three days in a mist chamber (20-22 °C and 100% relative humidity). Eighteen and 25 days after inoculation the plants were scored visually for the disease symptoms using a 1-9 scale (van Schoonhoven & Pastor-Corrales, 1987). Leaf DNA was extracted from the progenitors and from the resistant BC_nF₃ plants by a mini-prep procedure based on Doyle and Doyle (1990). Amplification reactions were according to Williams et al. (1990) using different primer sets.

Two F_{2,3} homozygous families were identified in each backcross all of them harboring the corresponding resistance gene (Figure 1).

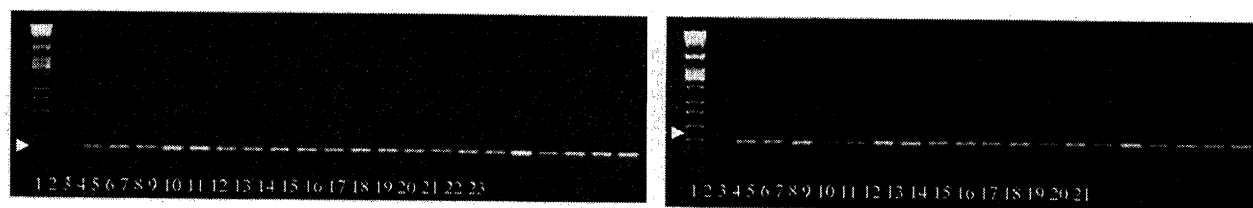


Figure 1A-B. Electrophoretic analysis of DNA amplification products obtained with primers RAPD OPE04 (A) (Ferreira et al. 2000) and SCARN2 (B) (Sartorato et al. 2000). Lines are as follows: 1, lambda DNA cut with *Eco*RI, *Bam*HI and *Hind*III; 2, Rudá; 3, Mar 2 (A) or Mexico 54 (B); 4-23, homozygous BC₁F₃ families (A) or 4-21, BC₂F₃ homozygous families (B). The arrows indicate RAPD marker OPE04 (A) and SCAR marker N2 (B).

Pairwise genetic distances between the recurrent parent and BC_nF₃ plants were determined (Table 1). A minimum of 20 RAPD primers were used for the analyses. The data show that the homozygous lines are still genetically distant from the recurrent parent, which would be expected

after only one or two backcrosses. However, the selection of the homozygous lines was based on the presence of the resistance gene and the phenotypic aspect of their grains are indistinguishable from that of Ruda. The lines which were genetically closer to Ruda will be characterized with those *P. griseola* races previously tested in the donor parents.

Table 1. Relative genetic distances between BCnF3 homozygous resistant lines and the recurrent (Ruda) and donor parents (Mexico 54 or Mar 2). The genetic distance between the recurrent and the donor parents was considered to be 1.00.

Family A1	Ruda	Mexico 54	Family B1	Ruda	Mar 2
9-2-117-92	0.500	0.733	139A-142-131	0.657	0.629
9-2-117-93	0.483	0.755	139A-142-132	0.685	0.615
9-2-117-94	0.727	0.657	139A-142-133	0.794	0.590
9-2-117-95	0.548	0.744	139A-142-134	0.757	0.652
9-2-117-96	0.600	0.651	139A-142-135	0.666	0.592
9-2-117-97	0.575	0.697	139A-142-136	0.636	0.703
9-2-117-98	0.548	0.744	139A-142-137	0.657	0.629
9-2-117-99	0.515	0.711	139A-142-138	0.638	0.607
9-2-117-100	0.531	0.727	139A-142-139	0.617	0.678
9-2-117-101	0.516	0.750	139A-142-141	0.558	0.700
Family A2			Family B2		
37-6-155-126	0.641	0.627	139A-120-156	0.695	0.666
37-6-155-127	0.710	0.625	139A-120-157	0.583	0.666
37-6-155-128	0.648	0.666	139A-120-158	0.541	0.684
37-6-155-129	0.675	0.658	139A-120-159	0.565	0.722
37-6-155-130	0.685	0.700	139A-120-160	0.545	0.777
37-6-155-131	0.685	0.700	139A-120-161	0.650	0.866
37-6-155-132	0.617	0.738	139A-120-162	0.521	0.736
37-6-155-133	0.666	0.682	139A-120-163	0.571	0.823
			139A-120-164	0.478	0.750
			139A-120-165	0.681	0.733

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